BOLD fMRI of Rat Retina at 11.7 Tesla

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INTRODUCTION BOLD fMRI has revealed layer-specific changes associated with gas challenges rat retina (1). Detecting visually evoked BOLD fMRI responses in the retina remain very challenging. The only report of BOLD fMRI of visual stimulation (drifting gratings) was on cat retina in 2002 at a spatial resolution of 485x485x2000 microns (2). Rat is a widely used animal model for retinal research. BOLD fMRI of visual stimulation in the rat retina has yet to be demonstrated. In this study, we described the challenges and solutions for performing BOLD fMRI of visual stimulation in the rat retina at 110x110x1000 microns. Visual stimuli employed diffuse achromatic light flickering at 8 Hz. This approach provides a means to evaluate functional changes in the rat retinas where many retinal disease models are readily available.

METHODS Male Sprague Dawley rats (200-300g) were anesthetized with 0.9~1.0% isoflurane, mechanically ventilated, and paralyzed with pancuronium bromide (3 mg/kg first dose, 1 mg/kg/hr, ip) (1,3). Visual stimulus (full field) was 8 Hz flickering achromatic light delivered via an optical fiber with a diffuser. MRI acquisitions were typically acquired with 10 epochs of (60s OFF and 30s ON) followed by 60s OFF, or 5 epochs of (60s OFF and 30s ON) followed by 60s OFF. MRI studies were performed on an 11.7-Tesla/16-cm magnet and a 77G/cm BGA9S gradient insert (Bruker, Billerica, MA). Rats were placed in a head holder consisting of ear and tooth bars. A custom-made small circular surface coil (ID~7 mm) was placed on the left eye. BOLD fMRI were acquired using single-shot gradient-echo, echo-planar imaging with inversion recovery contrast using TR = 3 s, TE = 13 ms, TI = 1.3 s, spectral width = 200 kHz, matrix = 90x90, FOV = 10x10 mm (110x110 μm) and a single 500 μm thick slice. Vitreous signal was suppressed using inversion recovery. Images were corrected for potential motion and drift before additional analysis. BOLD fMRI analysis employed cross-correlation analysis. In addition, the retina was linearized and aligned (4). Profiles across the retinal thickness of the baseline and stimulation periods were analyzed. Percent changes were tabulated for active pixels on the retina and ROI of the entire retina. Reported values were in mean ± SD.

RESULTS **Figure 1** shows the anatomical images of an animal without eye drops and one with eye drops. The ciliary muscles were pulled back. The pupil was dilated and remained dilated at the end of the MRI experiments. Before eye drop application, activated pixels in the retinas and in the ciliary muscles were consistently detected (data not shown). The animal was slide out of the scanner and eye drops were applied and slide back in without re-shimming. After eye drop application, activated pixels in the ciliary muscles were eliminated. Moreover, time-series movies of anatomical and functional MRI images showed that the pupils and ciliary muscles moved in correlation with stimulus onset before eye drops; these movements were markedly reduced or eliminated after the applications of the eye drops (data not shown). As negative controls to confirm that activated pixels were free of "correlation noises," fMRI data were compared with and without visual stimulation on the same animals using identical acquisition and analysis protocols. Without visual stimulation, no significantly activated pixels were detected at the same statistical threshold and BOLD changes of the whole-retina ROI were 0.08 ± 0.34% (mean ± SD, n = 6 trials on 5 rats), not statistically different from baseline (P > 0.05).

Figure 2 shows the BOLD fMRI activation map and the corresponding time course of a representative animal with application of atropine and phenylnephrine eye drops and optimized shimming to minimize signal dropout and distortion of the EPI. Activated pixels were predominantly localized to the retina without activated pixels in the pupils and ciliary muscles. The group-averaged percent changes from activated pixels in the retina was 5.0 ± 0.8 % (mean ± SD, n = 12 repeats on 6 rats, P < 0.05 compared with baseline). Data were also analyzed using an ROI of the entire retina. The group-average percent changes from the whole-retina ROI was 3.1 ± 1.1 % (±SD, n=12 repeats on 6 rats, P < 0.05 from baseline).

DISCUSSION This study demonstrates high-resolution BOLD fMRI of visual stimulation on the rat retina at 11.7 T at 110x110x1000 μm. High field improves signal-to-noise ratio, BOLD contrast and spatial resolution. Inversion recovery allows suppression of, and thus avoids contamination by, the strong vitreous signals. To verify that activated pixels were not a result of correlation artifacts from physiological or electronic noise, the same fMRI protocols and analysis were performed without visual stimulation. No significantly activated pixels were detected at the same threshold and % changes of the whole-retina ROI were within noise level when no visual stimulations were presented, demonstrating that the visual activation on the retina as detected by BOLD fMRI is genuine.

In conclusion, depth-resolved BOLD fMRI of the retina has the potential to complement existing optical imaging techniques and should have applications in studying retinal function in disease states, such as diabetic retinopathy, glaucoma, and retinal degeneration that are widely available in rodent models.

References 1. Cheng et al., PNAS 103, 17525 (2006). 2. Duong et al, IOVS 43, 1176 (2002). 3. Li et al., IOVS 50, 1824 (2009). 4. Cheng & Duong, Opt Lett 32, 2188 (2007).

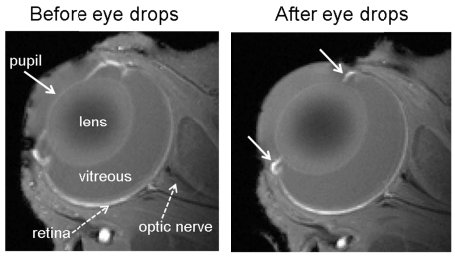

Figure 1. Anatomical images of the eye before and after eye drops (atropine and phenylnephrine) in the same animal. With eye drops, the pupil was dilated and the ciliary muscles, a ring of striated smooth muscles that controls accommodation, were pulled back.

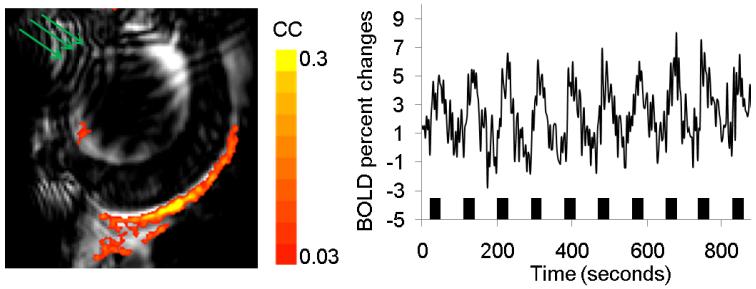

Figure 2. BOLD fMRI map overlaid on EPI and signal time course of visual stimulation of a rat retina at 11.7T.